Strain-specific PrP-res *in Vitro* Amplification by the PMCA Technique.

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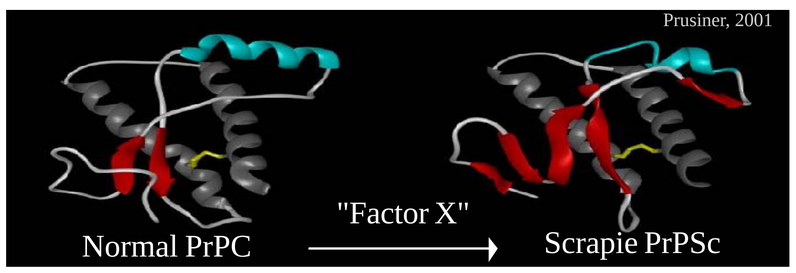
Foodborn Contaminants Research

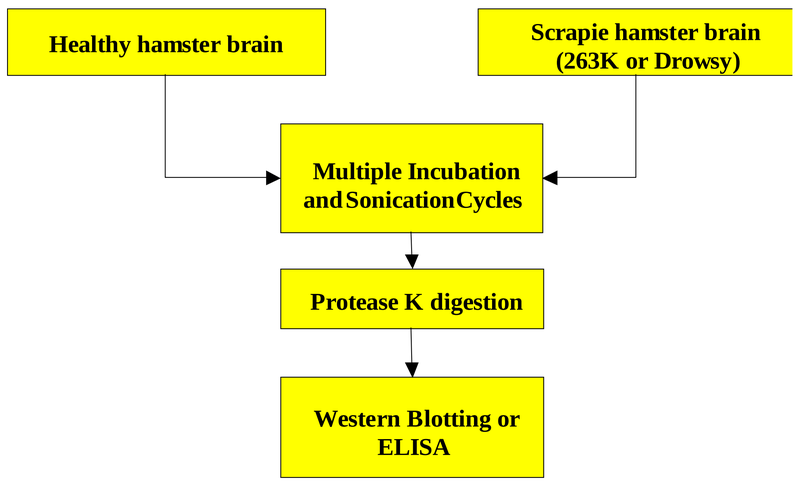
Our efforts focus on investigating the *in vitro* conversion of the normal cellular prion protein (PrP^{C}) to its infectious isoform (PrP^{Sc}) in order to understand the nature of the conversion.



We use the Protein Misfolding Cyclic Amplification (PMCA)[1,2] technique to study this phenomenon. PMCA is an *in vitro* method of converting PrP^{C} to PrP^{Sc} that is believed to mimics the *in vivo* conversion process. The resulting amplification of PrP^{Sc} by PMCA allows for the detection of otherwise undetectable amounts of PrP^{Sc}.

We used two well-characterized hamster-adapted prion strains (263K and drowsy (DY) in our investigations[3,4].

PMCA Protocol and PrP^{Sc} Detection



The 263K and DY strains have different incubation periods, clinical symptoms, biochemical, and physical properties. Both strains are post-translationally derived from hamster PrP^{C}. Each strain is a distinct conformer that self-propagates *in vivo* by converting PrP^{C} to its PrP^{Sc} conformer (either 263K or DY). Furthermore, each strain has a characteristic SDS-PAGE migration pattern after partial protease K (PK) digestion. The DY strain is more susceptible to PK digestion than the 263K strain.

Experimental

Sample preparation. 10% brain tissue homogenates contained a "complete" cocktail of protease inhibitors (Roche), 4mM EDTA, and 1% Triton X-100 in PBS buffer.

Cyclic amplification. Samples (50µl) were incubated at 37 °C. Sonication was performed every hour (300 W, 5s. pulse) in a 96 well sonicator.

Detection of PrP^{Sc}. PrP^{Sc} was denatured and detected by Western blot. We used a biotynilated 3F4 antibody - streptavidin-AP complex as a probe / reporter system for the Western blots.

Detection of PrP^{C}. PrP^{C} was detected by native ELISA. We used a new monoclonal antibody that was produced in our lab to capture PrP^{C} and the biotynilated 3F4 - streptavidin-HRP complex to detect it.

Results

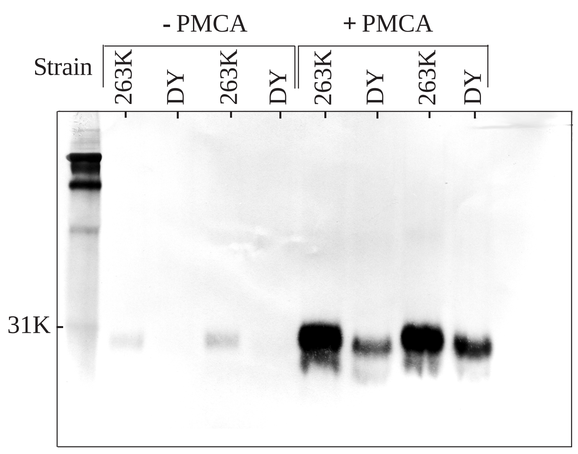


Fig. 1. Cyclic amplification of 263K and DY PrP^{Sc} strains. Brain homogenates from terminally ill hamsters were added to healthy 10% brain homogenate at a final dilution of 1:200. After 48 amplification cycles the samples were digested with protease K and analyzed by Western blot.

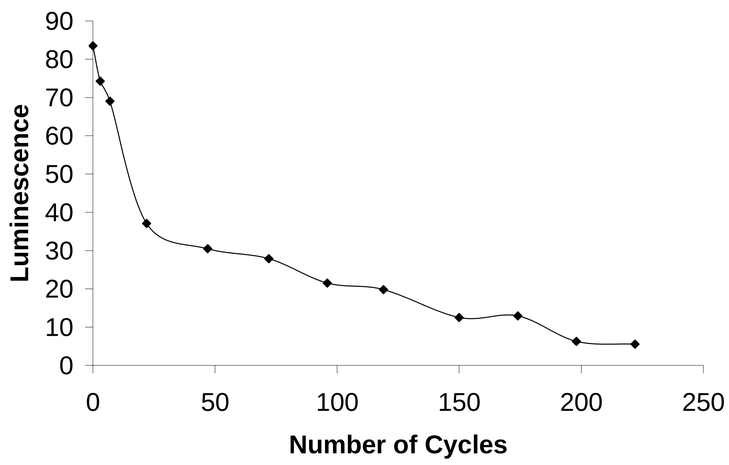


Fig. 2. Stability of PrP^{C} under PMCA conditions. 10% brain homogenate of healthy hamsters containing a "complete" cocktail of protease inhibitors (Roche), 4mM EDTA, and 1% Triton X-100 in PBS buffer was subjected to multiple cycles of incubation and sonication at 37 °C. The samples were analyzed by native ELISA.

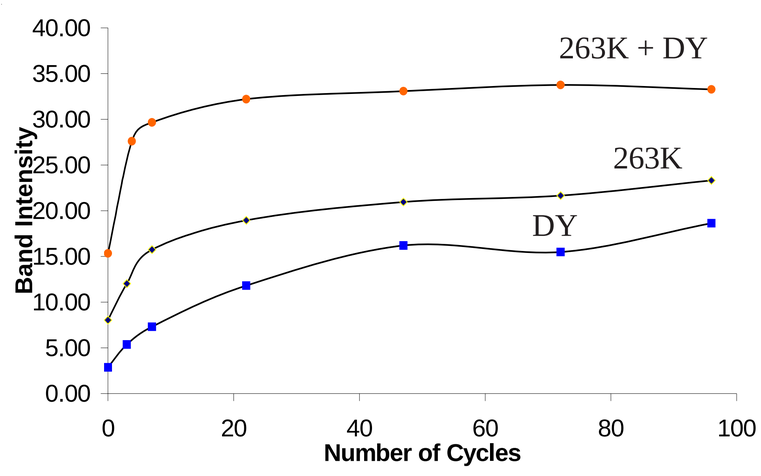


Fig. 3. Analysis of Western blots after PrP^{Sc} amplification by densitometry. The 263K, DY, and the mixture of 263K and DY underwent more than 50% amplification.

Conclusion

- Both the 263K and DY strains undergo significant amplification by the PMCA technique.
- The material amplified from the 263K and DY strains show distinct gel migration patterns (Fig. 1) that are characteristic of the progenitor strains. This suggests that PMCA amplified the distinct 263K and DY PrP^{Sc} conformers.
- The DY strain becomes more protease K resistant (Fig. 3) upon amplification, though it retains its distinct gel migration pattern. The reason for this result is unclear and need to be evaluated *in vivo*.
- Amplification of PrP^{Sc} slows down with the number of amplification cycles (Fig. 3). That coincides with the decomposition of PrP^{C} (Fig. 2). Better protease inhibitors would probably increase the stability of PrP^{C} and therefore increase the amplification factor.

References

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